JOURNAL OF BACTERIOLOGY, May, 1966 Vol. 91, No. 5

Pasteurella pestis: Growth Temperature, Virulence, and the Graded Response

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ABSTRACT

ROSENWALD, ALBERT J. (Fort Detrick, Frederick, Md.), AND RALPH E. LINCOLN. *Pasteurella pestis:* growth temperature, virulence, and the graded response. J. Bacteriol. 91:1693–1695. 1966.—A comparison of the virulence of *Pasteurella pestis* was made by the graded and quantal response methods. Both tests reflected the difference in virulence of cultures grown at three temperatures. Cultures grown at lower temperatures gave the most variable response in virulence tests, and cultures grown at higher temperatures were more virulent. Results from the graded response test were obtained more quickly and more economically than those from the quantal response test.

The virulence of *Pasteurella pestis* is measured most often by the quantal response (LD_{50}); however, the innate variability in this method, together with the variable response elicited by plague organisms, particularly those grown at 26 to 28 C, often results in data with wide confidence limits. The graded response [median time-to-death (MTD)] is another method of determining virulence, a method which does not appear to have been applied to *P. pestis*. A comparison of the quantal and graded response methods was made by Roth, DeArmon, and Lively (7), Fernelius et al. (2), Lincoln and DeArmon (5), and DeArmon and Lincoln (1). This report describes the use of the graded and quantal responses in comparing the virulence of cultures of *P. pestis* grown at different temperatures.

MATERIALS AND METHODS

Bacterial strain. P. pestis strain L-37, obtained from the Microbiological Research Dept., Porton, England, was used in these studies. It is virulent, and, in its present unselected state, carries in it a low level of avirulent cell types as determined by colony differentiation on hemin-agar and on magnesium oxalate-agar.

Cultural conditions. The components of the liquid growth medium were N-Z-Amine Type A, 3.0% (Sheffield Chemical, Norwich, N.Y.); yeast extract, 1.0% (Difco); K_2HPO_4, 0.4%; in tap water at *p*H 7.1 ± 0.1. Galactose (2%, final concentration) was added aseptically after sterilization. This medium was used at a volume of 25 ml in a 250-ml Erlenmeyer flask to which 1 ml of liquid culture was added as inoculum. Liquid cultures were shaken for 24 hr on a reciprocating shaker (100 3-inch strokes per minute) at the desired temperature of growth.

Viable cell counts were done by surface plating suitable dilutions of the bacteria on Difco Blood Agar Base medium (BAB) supplemented with 0.1% glucose and 0.04% sodium sulfite. The magnesium oxalate-agar (MGOX) was described by Higuchi and Smith (3). These two media were incubated, after inoculation, for 40 to 48 hr, the BAB at 26 C, and the MGOX at 37 C. The inoculated hemin-agar plates (4) were incubated for 96 hr at 26 C.

The diluent used for all purposes was composed of N-Z-Amine Type A, 0.5%; and NaCl, 0.5%; in distilled water at *p*H 7.2.

Virulence tests. The quantal response was determined by intraperitoneal injection of Swiss-Webster mice (16 to 20 g) with 0.2 ml of suitable dilutions of the culture. Six doses with 10 mice per dose covering the estimated range of 0.5 to 16.0 organisms were used in each titration, and, after 10 days, the LD_{50} was calculated according to the method of Litchfield and Wilcoxon (6).

The graded response was determined by intraperitoneal challenge of part of the same group of mice with 0.5 ml of a dilution of the culture. Doses spaced a log apart, as given in Table 2, with 10 mice per dose, were used in each titration. As a toxin control, culture supernatant fluid, sterilized by passage through an ultrafine fritted-disc filter, was diluted to the least dilution used in the titration and used to challenge mice. The mice were checked hourly and then at more frequent intervals when deaths began to occur. (No deaths occurred in the toxin control.) The elapsed period between time of challenge and the average of the times-to-death of the fifth and sixth animals was taken as the MTD.

RESULTS

Typical data in the comparison of the graded and quantal response of mice to *P. pestis* are shown in Tables 1 and 2. Table 1 shows: (i) total viable count of the population tested, (ii) the percentage of this population that grew on MGOX agar, (iii) the percentage that was pigmented on hemin-agar, and (iv) the LD_{50} of these cultures. The data show that cell yield and growth temperature share an inverse relationship. A more direct relationship is indicated between growth temperature and virulence, with the higher temperatures favoring virulence in the organism. Growth on MGOX agar is comparable at all three temperatures, as is the percentage pigmented on hemin-agar.

Table 2 shows dose and MTD for the graded response test. The results are presented graphically in Fig. 1, which shows that determinations of virulence of *P. pestis* can be made reliably by the graded response in from 10 to 40 hr, far less time than the 10 days ordinarily required for the quantal response. As with the quantal response test, determinations by the graded response show the greatest virulence and the least variability in response in cultures grown at higher temperatures. Variability of response of cultures grown at 26 C is reflected not only in wider confidence limits in the quantal test, but also in the degree of scatter of the points on the slope for the graded test. This variability is of some importance, since *P. pestis* cultures are commonly grown at 26 to 28 C, achieving a greater cell yield in the same incubation period than similar cultures grown at 37 C. The 26 C cultures also retain their virulence through more generations than those grown at 37 C.

After a partial slope has been established by the graded test for a particular set of experimental conditions, virulence level may be determined with a minimum of five animals. This reduction in number of animals required for the graded test, as well as the shorter holding time, makes this test considerably more economical than the quantal test.

TABLE 1. *Viable-cell count, per cent differential response on indicator media, and LD_{50} of Pasteurella pestis grown at 26, 31, or 37 C*

Growth temp	Total viable cell count (10^9/ml)	Count on MGOX agar*	Pigmented on hemin-agar*	LD_{50} (95% confidence limits)
C				
26	346	1.21	74.6	6.7 (3.1–14.6)
31	202	3.96	75.0	5.5 (3.0–9.9)
37	45.6	2.85	73.3	0.6 (0.3–1.3)

* Expressed as per cent of total.

TABLE 2. *Dose and median time to death of mice challenged with Pasteurella pestis grown 26, 31, or 37 C*

Growth temp					
26 C		31 C		37 C	
Log dose	MTD	Log dose	MTD	Log dose	MTD
	hr		*hr*		*hr*
9.238	<6.8	9.004	<6		
8.238	12.4	8.004	11.6	8.358	8.0
7.238	30.7	7.004	20.6	7.358	10.9
6.238	43.8	6.004	26.8	6.358	19.5
5.238	39.8	5.004	30.1	5.358	27.2
4.238	41.9	4.004	39.3	4.358	31.3
3.238	47.8	3.004	45.0	3.358	39.7
2.238	58.3	2.004	56.5	2.358	45.6
1.238	*	1.004	91.1	1.358	56.0
0.238	*	0.004	*	0.358	113.0

* Partial response—not calculable.

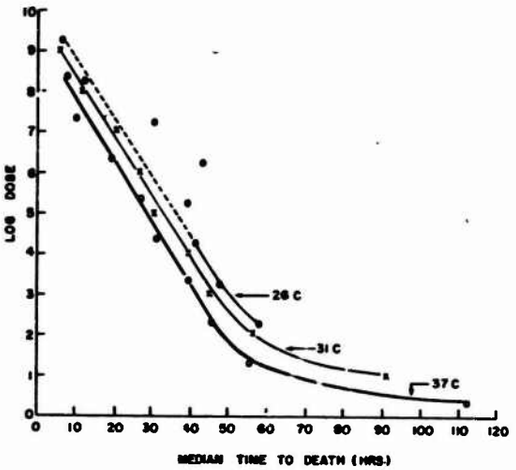

FIG. 1. *Dose and graded response of mice to Pasteurella pestis grown at different temperatures.*

ACKNOWLEDGMENT

We express our appreciation for the excellent technical assistance of Charles C. Wigington.

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